DNA high-risk HPV, mRNA HPV and P16 tests for diagnosis of anal cancer and precursor lesions: a systematic review and meta-analysis

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Summary

Background Anal cancer prevention has two critical points: the incidence rate is several fold higher for some groups, such as people living with human immunodeficiency virus (HIV) and men who have sex with men (MSM), and there is not a well-defined guideline for its screening. This systematic review evaluates the accuracy of DNA HRHPV (high-risk human papillomavirus), mRNA HPV, DNA HPV16 isolated and p16 staining biomarkers in anal canal smears for identifying anal intraepithelial neoplasia (AIN) 2 or 3, summarised as anal high-grade squamous intraepithelial lesions (aHSIL), and cancer.

Methods We searched the MEDLINE, Cochrane Library and Embase electronic databases as well as Grey literature to identify eligible papers published up to 31st July 2022. This systematic review and meta-analysis included observational studies comparing biomarker tests to histopathology after HRA (High-resolution Anoscopy) as a reference standard. We (ACM, TF) analysed studies in which patients of both sexes were screened for anal cancer using DNA HRHPV, mRNA HPV, DNA HPV16 and/or p16 biomarkers. The analysis was performed in pairs, for instance AIN2 or worse (AIN2+) vs. AIN1, HPV infection and normal (AIN1-). PROSPERO CRD42015024201.

Findings We included 21 studies with 7445 patients. DNA HR HPV showed a higher sensitivity 92.4% (95% CI 84.2–96.5), specificity 41.7% (95% CI 33.9–44.9) and AUC 0.67, followed by the mRNA HPV test, with a sensitivity 77.3% (95% CI 73.2%–80.9%), specificity 61.9% (95% CI 56.6–66.9) and AUC 0.78. DNA HPV16 showed higher specificity 71.7% (95% CI 55.3–83.8), followed by p16 test, 64.1% (95% CI 51.0–75.4); Sensitivity of DNA HPV16 was 53.3% (95% CI 35.4–70.3) and AUC 0.69, while p16 had a sensitivity of 68.8% (95% CI 47.9–84.1) and AUC 0.74. Subgroup analysis of MSM with HIV, with 13 studies and 5123 patients, showed similar accuracy, with a bit higher sensitivities and lower specificities. Considering the measure of the total between-study variability, mRNA HPV tests showed the smallest area of the 95% prediction ellipse, 6.0%, influenced by the low logit sensitivity, 0.011. All other groups of tests exceed 50% prediction ellipse area, which represent a high heterogeneity.

Interpretation Our findings suggested that DNA HR HPV can be a useful tool for screening for aHSIL and anal cancer if followed by biomarker with a higher specificity. As an isolated test, mRNA HPV had better performance.

Funding There was no funding source for this study.

Keywords: mRNA HPV; DNA HR HPV; p16; AIN; Anal cancer; Diagnostic systematic review

Introduction

In 2020, there were 50,865 newly diagnosed cases of anal cancer worldwide and this number is predicted to increase to 78,000 in 2040, according to "Cancer Tomorrow" on the Global Cancer Observatory.[1,2] The most common histological subtype is squamous-cell

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Research in context

Evidence before this study

The present systematic review assesses the accuracy of DNA HR HPV, mRNA HPV, DNA HPV16 isolated and p16 tests in anal canal smears to identify anal high-grade squamous intraepithelial lesions (HSIL) and cancer, compared to histopathology. We searched the databases as well as reference lists and Grey literature up to 31st July 2022. The following terms were used: "anal cancer", "anal dysplasia", "squamous intraepithelial lesion", "anal intraepithelial neoplasia", "AIN", "screening", "DNA HR HPV", "p16" and "mRNA HPV".

We analysed studies that included people who were screened for anal cancer in secondary settings. Whole patients were submitted for HRA and, whenever possible, only biopsied cases were included. The exclusion criteria were index tests were performed using tissue fragments and not cytology specimen.

Three hundred thirty-three records were screened. Twenty-one primary studies, with 7445 patients, were included in the analyses. Of the main analysis, 18 studies reported the major outcome, AIN1- vs. AIN2+, and 8 studies reported the outcome as normal vs. AIN1+. In addition, 12 studies, with 5038 patients, were men sex men (MSM) with HIV and were subject to separate analyses.

Added value of this study

The present systematic review substantiates biomarker accuracies for anal HSIL (AIN2+) and cancer screening and confirmed that anal lesion screening follows the same trends as cervical lesion screening. DNA HR HPV tests presented a high sensitivity but a very low specificity; mRNA HPV tests presented a better area under the Curve (AUC) and p16 presented similar values for sensitivity and specificity. We concluded that DNA HR HPV can be a useful tool for screening for aHSIL and anal cancer if followed by a biomarker with a higher specificity. mRNA HPV had better performance, for both the "whole group" and "MSM with HIV".

Implications of all the available evidence

This systematic review has shown the accuracy of DNA HR HPV, mRNA HPV, DNA HPV16 isolated and p16 staining biomarkers for anal cancer screening and allows its implementation on a larger scale. However, additional prospective studies are necessary to establish the efficacy of cancer prevention and cost-effectiveness, in addition to larger studies with women with HPV, leading to the definition of screening guidelines.

carcinoma (SCC) with an annual incidence of 0.5–2.0 in 100,000.[3] Anal cancer prevention has two critical points: the incidence rate is several fold higher for some groups, such as people living with human immunodeficiency virus (HIV) and men who have sex with men (MSM), and there is not a well-defined guideline for its screening.[4] According to a previous meta-analysis, the incidence of anal cancer is 45.9 per 100,000 for MSM living with HIV, and 5.1 per 100,000 for MSM without HIV.[5] Early diagnosis is possible if anal high-grade squamous intraepithelial lesions (HSILs) are identified before the development of anal squamous cell cancer. Screening tests for anal cancer could make this disease one of the most easily preventable malignant tumours.[6] Currently, screening is not well-defined since was not clear how much untreated high-grade lesions could progress to invasive cancer. However, the recent publication of the first ANCHOR study results, performed with 4446 persons living with HIV, showed that among participants with biopsy-proven anal HSIL, the cumulative incidence of progression to anal cancer at 48 months was 0.9% in the treatment group and 1.8% in the active-monitoring groups.[7]

Usually, cervical cancer prevention is used as a model because it is a very similar disease to anal cancer. They are both associated with HPV (human papillomavirus), predominantly HPV 16. HPV DNA (deoxyribonucleic acid) is detected in more than 88% of anal cancers and more than 95% of anal HSIL[8] while is detected in nearly 100% of cervical cancers.[9] Both are preceded by precursor lesions, after HPV infection, going from normal to low grade or high grade, and have similar histopathologic manifestations.[8,10] Cytology, widely used for cervical cancer screening worldwide, has shown accuracy ranging from 77.3% to 85.0% for sensitivity and specificity from 43.2% to 55.5% in previous metanalyses, considering "ASC-US-positive cytology or higher" for the detection of "AIN2 or higher".[11,12] Actually, cytology can be considered an improvement in anal cancer screening, but it does not have an excellent sensitivity for triage or a good specificity that could avoid unnecessary High Resolution Anuscopy (HRA) with biopsy.

Given consensus regarding the causal role of high-risk human papillomavirus (HR HPV) in the development of anal cancer,[13] DNA HR HPV, mRNA (messenger ribonucleic acid) HPV and p16 tests should be considered for anal cancer screening, as their accuracy has been shown for cervical cancer.[6]

The present systematic review assesses the accuracy of DNA HR HPV, mRNA HPV, DNA HPV16 isolated and p16 tests in anal canal smears to identify anal high-grade squamous intraepithelial lesions (HSIL) and cancer. The prespecified hypothesis, based on cervical cancer screening performance,[14,15] is that DNA HR HPV presents a high sensitivity and mRNA HPV and p16

biomarkers exhibit acceptable accuracy and high specificity for the detection of high-grade squamous intraepithelial lesions or anal cancer.

Methods

Study design

We performed a systematic review according to a prospective protocol using PRISMA2 statement guidelines,[16] and SAGER guidelines (Sex and Gender Equity in Research).[17] This review protocol is registered at PROSPERO (International prospective register of systemic reviews, http://www.crd.york.ac.uk/prospero; CRD42015024201).

To define the review question, the PICO strategy[18] was applied: the Patients (P) were women and men who were screened for anal cancer in secondary settings; the intervention (I) was "DNA HR HPV", "p16", "DNA HPV16" and "mRNA HPV" diagnostic tests; the comparison intervention (C) was histopathological; and the clinical outcome of interest (O) was high-grade squamous intraepithelial lesion or anal cancer.

Research Ethics Committee (REC) approval is not typically required for a systematic review, as it involves analyzing and synthesizing existing data rather than collecting new data from human participants. In the context of a systematic review, researchers typically adhere to ethical principles such as transparency, rigor, and ensuring the privacy and confidentiality of the data sources used.

Identification of studies

The Medline via Pubmed, Lilacs via VHS, Cochrane Library and Embase via Elsevier electronic databases, as well as Grey literature published until 31st July 2022. The following medical subject headings (MeSH) and text words were used for the search: "anal cancer", "anal dysplasia", "squamous intraepithelial lesion", "anal intraepithelial neoplasia", "AIN", "screening", "DNA HR HPV", "p16" and "mRNA HPV". No language restrictions were applied. The reference lists of all available primary studies were manually searched to identify additional relevant citations.

Study selection

As no randomised studies were identified, this review focused on observational studies in which the "DNA HR HPV", "p16", "DNA HPV16" and "mRNA HPV" diagnostic tests were compared to a histopathological reference standard. All included studies were cross-sectional or cohort studies (cohort were only included if biomarkers and histopathology were available at baseline, to characterise cross-sectional data).

The title, abstract and full-texts were screened by two independent investigators (TA, ACM). Disagreements regarding study inclusion or exclusion were initially resolved by discussion. When disagreements persisted, a third reviewer was consulted (MIR).

Patients

We analysed studies that included women and men who were screened for anal cancer in secondary settings, that is, testing performed in Infectious Disease Centres, after someone has had an abnormal result by cytology or patient with coloproctological disease. The criteria for inclusion in each study required diagnosis of index and reference tests. All patients were submitted for HRA and, whenever possible, only biopsied samples were included. We considered discriminating by sex and gender whenever available. These variables were subsequently considered in the sensitivity analysis.

Index tests

The index tests were "DNA HR HPV", "p16" and "mRNA HPV" assays from a sampling of anal smear, collected from the cytology specimen. Positive and negative reads were assigned according to the cut-off points proposed by the manufacturers.

The exclusion criteria were index tests were performed using tissue fragments and not cytology specimen. Studies in which all specimens were diagnosed as cancer were excluded, since there were no false positive or true negative.

Reference standard

The reference test was histologic evaluation of tissue in paraffin-embedded sections using the Bethesda System classification.[19]

Data extraction

This study was independently reviewed by two investigators (TA, ACM). Disagreements with regard to study inclusion or exclusion were initially resolved by consensus. When consensus was not attained, disagreements were resolved by a third reviewer (MIR).

A form designed according to the study profile was prepared and applied, for qualitative and quantitative analysis and sensitivity analysis.

In addition to "last author name" and "year of publication", which composed the study identification, the following were extracted; 1) about patients: healthcare context, HIV status, sexual behaviour if discriminated, age, percentage of women/men, HAART use (Highly Active Antiretroviral Therapy) in studies with "people living with HIV"; 2) methodological study design, described data about blinding and interval between indices and reference test; 3) reference test: sample quantitative data (number of "benign" and "malign" in histopathological, percentage of biopsied cases; 4) about indices tests: brand and techniques and 5) TP, FP, FN and TN for each test, applying two cut-off: AIN1- vs. ANI2+ and normal vs. AIN1+.

Assessment of methodological quality

Methodological quality assessment of studies for diagnostic accuracy was performed according to criteria from the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2). These criteria assess the quality of the included studies in terms of risk of bias and concerns regarding applicability over four domains.[20]

The methodological quality of the included studies was assessed independently by the same investigators who performed data extraction. (TA, ACM, MIR).

Statistical analysis

A 2 × 2 contingency table was constructed for each selected study. Rates were calculated as true positive (TP), false positive (FP), true negative (TN), and false negative (FN). When any cell containing "0" was present in the contingency table, 0.5 was added to all cells in all studies to adjust calculations. Dichotomization of the contingency tables was performed by defining two categories: (1) AIN2 or worse vs. AIN 1 and normal (main analyses) and (2) AIN 1 or worse vs. normal.

For all studies, we calculated the true-positive rate (TPR; sensitivity), specificity, false-positive rate (FPR; 1—specificity), and the diagnostic odds ratio (DOR). The DOR, which relates to different combinations of sensitivity and specificity, was calculated by (sensitivity/(1-specificity))/((1-sensitivity)/specificity)). A DOR >1 indicated that the assay had discriminative power. The DOR describes the odds of the positive test results in participants with disease compared with the odds of positive test results in those without disease. Bivariate analysis was used to calculate pooled estimates of sensitivity, specificity, and DOR with 95% confidence intervals (CIs) for summary estimates.[21] The bivariate model preserves the 2-dimensional nature of the diagnostic data by analyzing the logit transformed sensitivity and specificity of each study in a single model and considers both within study and between-study variability, in contrast to the Littenberg and Moses method, which departs from a fixed effects model.

To analyse publication bias, Deek's test was applied and inverted funnel plots of the logarithmic odds ratio (OR) of individual studies were plotted against the sample size. The robustness of the results was tested by repeating the analysis with a different statistical model (random effects model). The meta-analysis was performed using STATA® 17 and Metadisc 2.0 software.

Investigations of heterogeneity

If sufficient studies were available, we intended to address the sources of heterogeneity by adding variables to the meta-analysis.

We assessed.

1. Difference in study population: by sex and sexual behavior, men of different genders pooled in MSM and MSW (Men sex Women).
2. Difference in study population: people living with HPV, HIV and immunosuppressive disease.

However, we did not perform some of the planned analyses due to insufficient information about relevant subgroups.

Heterogeneity quantitative analysis was performed using metadisc to measure the total between-study variability, by identifying Logit variances (sensitivity and specificity), the area of the 95% prediction ellipse and bivariate I^2.

Sensitivity analyses

We performed sensitivity analyses to validate the credibility of outcomes. We conducted a meta-analysis omitting.

1. Studies at moderate risk of bias in QUADAS-2 domains.
2. Studies estimated as outliers based on the forest plots.

And discerning.

3. By complete verification of the reference test or partial verification.
4. By different assays (techniques/brands) in the same "group tests".

Role of the funding source

There was no funding source for this study.

All authors had full access to all the data in the study and accept responsibility for the decision to submit for publication.

Results

Study identification and eligibility

Among the 332 studies identified from electronic database searches and reference lists, we excluded 275 published studies through title and abstract screening (Fig. 1). The complete strategy is available in Supplement 1. Fifty-seven full-text studies were then retrieved. Of those, 36 studies were excluded after further scrutiny. A complete list of excluded studies with justification is available in Supplement 2.

Study descriptions

Twenty-one primary studies were included[22–42] in the analyses. Of the main analyses, 20 studies[22–28,30–42] reported the major outcome, AIN1- vs. AIN2+, and 10 studies[29,33,35,37,40–42] reported Normal vs. AIN1+. A total of 7445 patients met the criteria for inclusion and were analysed. The main characteristics of the included studies are shown in Table 1. Table 2 shows the sum contingency tables regarding whole sample and men sex men (MSM) living with HIV applied for AIN1- vs. AIN2+, as accuracy (sensitivity, specificity, AUC, DOR and sum contingency

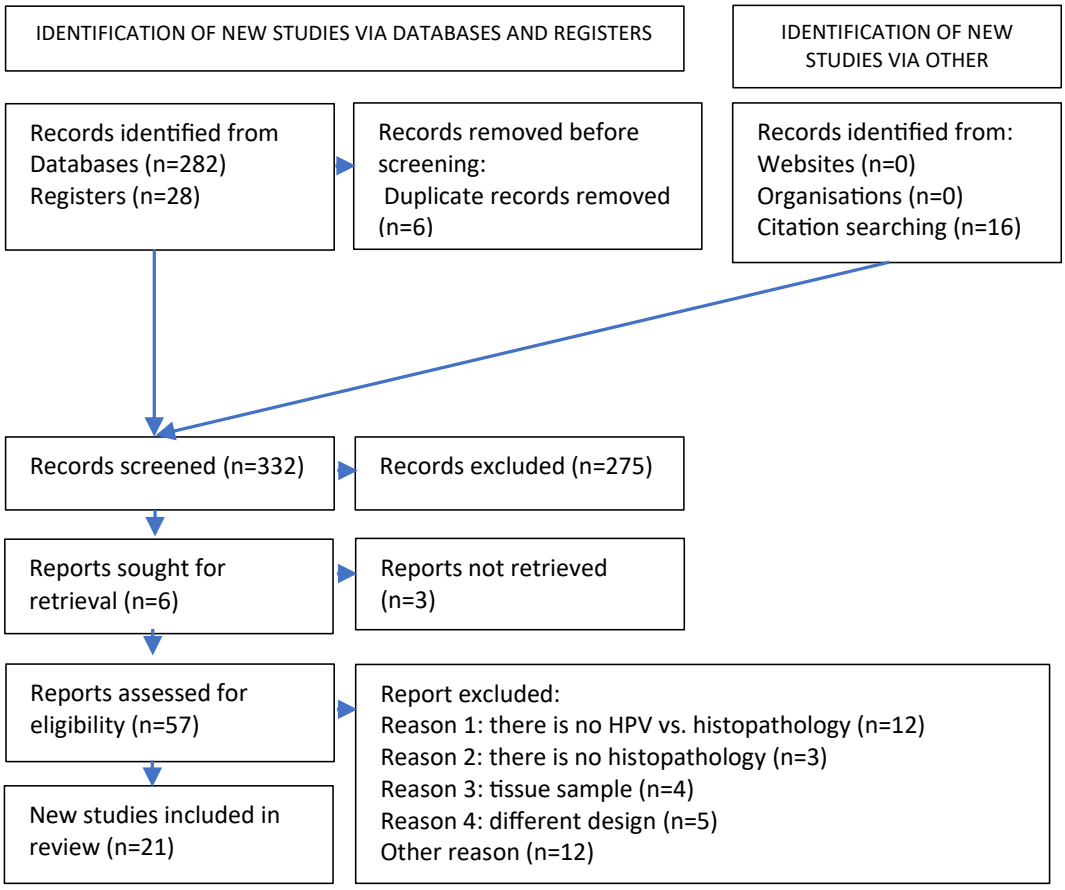

Fig. 1: **PRISMA flowchart of search strategy results.**

tables) by biomarker. The contingency tables per study may be requested from the authors. Template data collection form is available with authors.

Sample descriptions

Fifteen studies included participants below 35 years-old, although eighteen had a mean age over 35, considering those who informed it. Most were MSM living with HIV (5123/7445 participants). All studies reported sex proportion and in six studies in which both sexes were included, in five there was a small proportion of women (9.6%–28%).[23,30,33,36,41] None of 21 studies showed information about gender, i.e., identification in female, male and gender-diverse people.[17]

In the "people living with HIV" group at a minimum 74.6% of patients were using HAART (Highly Active Antiretroviral Therapy). Analyses of other high-risk subgroups such as "MSM regardless HIV status", "people living with HIV regardless gender" and "women with HPV" were not possible since few studies were available. Three studies included women living with HIV and enabled an HR HPV DNA test analysis, although it was a small sample.[22,26,30]

Quality assessment

QUADAS-2 was performed considering the following categories: patients, indices and reference tests and "flow and timing", as summarised in Fig. 2. For patient selection, one study was considered inadequate because it was a cohort of ASC-US cytology and had 8% of exclusions caused by paucicellularity, introducing punctual "moderate concern" risk of bias.[36] For the index and reference test, most studies did not mention blinding of the pathologists and were classified as "unclear". However, as all other criteria were fine, it was considered "low concern" of risk of bias. In addition, all included studies used a histopathological test as a reference, and the index tests were clearly cited. Therefore, "concern" with these items was low. For flow and timing, in four of twenty-one primary studies the verification of the histopathological examination was partial, that is, people with normal anoscopy were not biopsied, as shown in Table 1 and they were classified as having an "unclear risk of bias" and classified as "moderate concern" risk of bias.[24,25,27,28] In cohort studies, we considered baseline results, namely, index and reference tests collected at an interval considered adequate—up to 2 months between

Author, year	Country	Inclusion criterion	Age Mean (range)	Sex m/w	Study design	N total	N AIN2+	Biopsied %	DNA HRHPV test (if present)	mRNA HPV test (if present)	p16 test (if present)
Baranovski et al., 2012	USA	HIV-infected women	40 (22–57)	0/99	cohort	99	8	(All HRA)	HC2	–	–
Bertisch et al., 2013	Switzerland	All HIV-infected (cases: only cancer)	>25	132/23	nested cohort	155	41	100	Xmap	–	–
Burgos et al., 2017	Spain	HIV-infected MSM	40.8 (33–47)	574/0	cohort	574	77	(All HRA)	CLART2	–	–
Castle et al., 2013	USA	HIV-infected MSM	52 (33–79)	334/0	cross-sectional	334	62	(All HRA)	LINEAR ARRAY	PreTect HPV-Proofer	–
Chiao, 2020	USA	HIV-infected women	50 (44–55)	0/100	cohort	229	60	100	HC2	Aptima	–
Clarke et al., 2019	USA	HIV-infected MSM	53 (26–79)	359/0	cohort	359	255	73	Cobas 4800	PreTect HPV-Proofer	CINtecPLUS
Clifford et al., 2018	France	HIV-infected MSM	51 (45–56)	502/0	cross-sectional	502	51	44.8	Cobas 4800	–	CINtecPLUS
Etienney et al., 2012	France	hemorrhoidectomy and/or fissurectomy	47.3 (21–81)	147/153	cross-sectional	300	10	100	PCR	–	–
Gaisa et al.,	USA	Patients of ID Service	45 (34–54)	1661/176	cross-sectional	1837	756	99	Cobas 4800	–	–
Jin et al., 2017	Australia	MSM HIV-infected and not infected	49 (35–79)	500/0	cross-sectional	500	196	100	Cobas 4800	NucliSENS easyQ HPV	CINtecPLUS
Kimura et al., 2021	Brazil	Asymptomatic patients of ID Service	49.5 (38–60)	262/102	cross-sectional	364	61	100	Abbott RT HR HPV	–	–
Hildalgo-Tenorio, 2017	Spain	HIV-infected MSM	36.7 (25–47)	319/0	cross-sectional	319	44	100	LINEAR ARRAY	–	–
Pankam, 2017	Thailand	MSM HIV-infected and not infected	29 (24–36)	95/0	cross-sectional	32	22	100	LINEAR ARRAY	–	–
Phanuphak et al., 2013	Thailand	MSM HIV-infected and not infected	28.8 (21–35)	246/0	cohort	246	34	100	LINEAR ARRAY	HPV OncoTect	p16INK4a
Pichon et al., 2019	France	All - abnormal cytology	46.5 (19–70)	19/3	cohort	22	5	100	CLART2	–	CINtecPLUS
Sahasrabuddhe et al., 2013	USA	HIV-infected MSM	53 (26–79)	342/0	cross-sectional	342	104	100	LINEAR ARRAY	–	–
Salit et al., 2010	Canada	HIV-infected MSM	44.4 (39–50)	400/0	cross-sectional	400	98	94.6	HC2	–	–
Sendagorta et al., 2015	Spain	HIV-infected MSM	42 (33–50)	101/0	cross-sectional	101	47	100	CLART	NucliSENS EasyQ	–
Serrano-Villar et al., 2017	Spain	HIV-infected MSM	39 (29–49)	230/0	cross-sectional	230	68	100	–	–	CINtecPLUS
Walts et al., 2006	USA	Samples tissues from surgical pathology files	45.6 (19–72)	68/10	cross-sectional	78	41	100	–	–	p16 INK4a
Wentzensen et al., 2012	USA	HIV-infected MSM	53 (26–79)	274/0	cross-sectional	274	78	80.7	Cobas 4800	PreTect HPV-Proofer	CINtecPLUS

**: Not informed or absent; N: number; DNA: deoxyribonucleic acid; HR: High Risk; mRNA: messenger ribonucleic acid; HRA: High Resolution Anoscopy; ID: Infectious Disease; HPV: Human papillomavirus; HIV: Human immunodeficiency virus; MSM: Men Sex Men; AIN: Anal intra-epithelial neoplasia; PCR: Polymerase Chain Reaction.

Table 1: **The main characteristics of the included studies.**

Test All	DNA HR HPV% (IC 95%)	mRNA HPV% (IC 95%)	HPV 16% (IC 95%)	p16% (IC 95%)
Sensitivity	92.4 (84.2–96.5)	77.3 (73.2–80.9)	53.3 (35.4–70.3)	68.8 (47.9–84.1)
Specificity	41.7 (33.9–44.9)	61.9 (56.6–66.9)	71.7 (55.3–83.8)	64.1 (51.0–75.4)
DOR	8.7 (4.6–16.2)	5.52 (4.2–7.1)	2.88 (1.28–4.48)	3.93 (1.12–6.74)
AUC	0.67 (0.63–0.71)	0.78 (0.74–0.82)	0.69 (0.64–0.72)	0.74 (0.70–0.77)
TP	1768	466	230	448
FP	3172	544	649	597
FN	144	142	205	138
TN	1724	898	1394	849
N total	**6798**	**2050**	**2478**	**2032**
Studies	**20**	**7**	**6**	**8**
MSM HIV+				
Sensitivity	96.8 (89.2–99.1)	79.0 (74.0–83.0)	60.0 (54.0–65.0)	76.0 (49.0–91.0)
Specificity	32.1 (26.3–38.6)	59.0 (52.0–65.0)	67.0 (54.0–79.0)	65.0 (52.0–76.0)
DOR	14.13 (4.34–45.95)	5.0 (4.0–8.0)	3.0 (2.0–6.0)	6.0 (2.0–17.0)
AUC	0.55 (0.51–0.60)	0.80 (0.76–0.83)	0.62 (0.57–0.67)	0.73 (0.69–0.77)
TP	1315	269	191	249
FP	2550	350	574	405
FN	68	72	131	87
TN	1105	509	940	575
N total	**5038**	**1200**	**1836**	**1316**
Studies	**12**	**5**	**5**	**5**

MSM (Men Sex Men); HIV: human immunodeficiency virus; AIN: anal intraepithelial neoplasia; CI: Confidence interval; DOR: diagnostic odds ratio; AUC: area under the curve; TP: true positive; FP: false positive; FN: false negative; TN: true negative. Outcomes: AN1- vs. AIN2+.

Table 2: **Accuracy of DNA HR HPV, mRNA HPV, DNA HPV16 and p16 for detection of Anal Intraepithelial Neoplasia (AIN2+) in histopathological, Pooled and discerning by subgroup.**

cytology and histopathology samples. All studies were classified as "adequate interval".

Accuracy of DNA HR HPV

The accuracy of the DNA HR HPV test is presented in Table 2.[22,24–39,41,42]

The forest plot of main analysis (DNA HR HPV test, "all together" patients) is shown in Fig. 3 and SROC curve in Fig. 4. In this systematic review, five main tests were identified, the most frequent: 5 studies COBAS 4800® (Roche—PCR-RT); five, Linear Array HPV Genotyping® (Roche)–PCR and hybridization); three, CLART® 1 or 2 (Genomica—PCR); and three, Hybrid Capture® 2 (QIAGEN - liquid hybridization). When it was a genotyping technique, all HR genotypes were considered together.

Accuracy of mRNA HPV

The accuracy of HPV mRNA tests is presented in Table 2.[25–27,32,35,39,42]

Different techniques are available, based on the identification of mRNA HPV transcription, mainly of the E6 and E7 oncogenes. In this systematic review, four tests were identified: three studies applied PreTect HPV-Proofer® (NorChip AS), a real-time multiplex assay that uses nucleic acid sequence-based amplification (NASBA), a sensitive transcription-based amplification system (TAS) for the specific in vitro replication of mRNA; and two NucliSens EasyQ HPV® (BioMérieux) that is based on the original PreTect Proofer assay with the addition of the NucliSENS hardware platform and the software for NASBA measurements and data analysis, both identifying the same five most frequently recognised HPV types.[43] Only one study used OncoTect® (IncellDxTM, Inc.), which combines two techniques, called in situ hybridization and flow cytometry[35] and one study used Aptima® (Hologic Gen-Probe, San Diego, CA, USA), a target amplification assay utilizing transcription-mediated amplification (TMA) for qualitative detection of viral polycistronic E6/E7 mRNA from 14 high-risk HPV types.[44]

Accuracy of p16 staining test

The accuracy of 16 staining is shown in Table 2.[27,28,32,35,36,40–42]

p16 ou p16^{INK4a} is a protein that slows cell division thereby acting as a tumour suppressor and that can be overexpressed in cervical or anal cells.[45] Six studies applied the *CINtec PLUS* assay® (Roche Diagnostics),[27,28,32,36,40,42] a dual-stain immunocytochemical test which that detects p16 and Ki-67 proteins and four studies applied p16 immunohistochemical staining from others manufactures, one of them associated with Ki-67.

Accuracy of the HPV16 DNA test

The accuracy of DNA HPV16 discriminated in Table 2.[23–25,28,33,35,42]

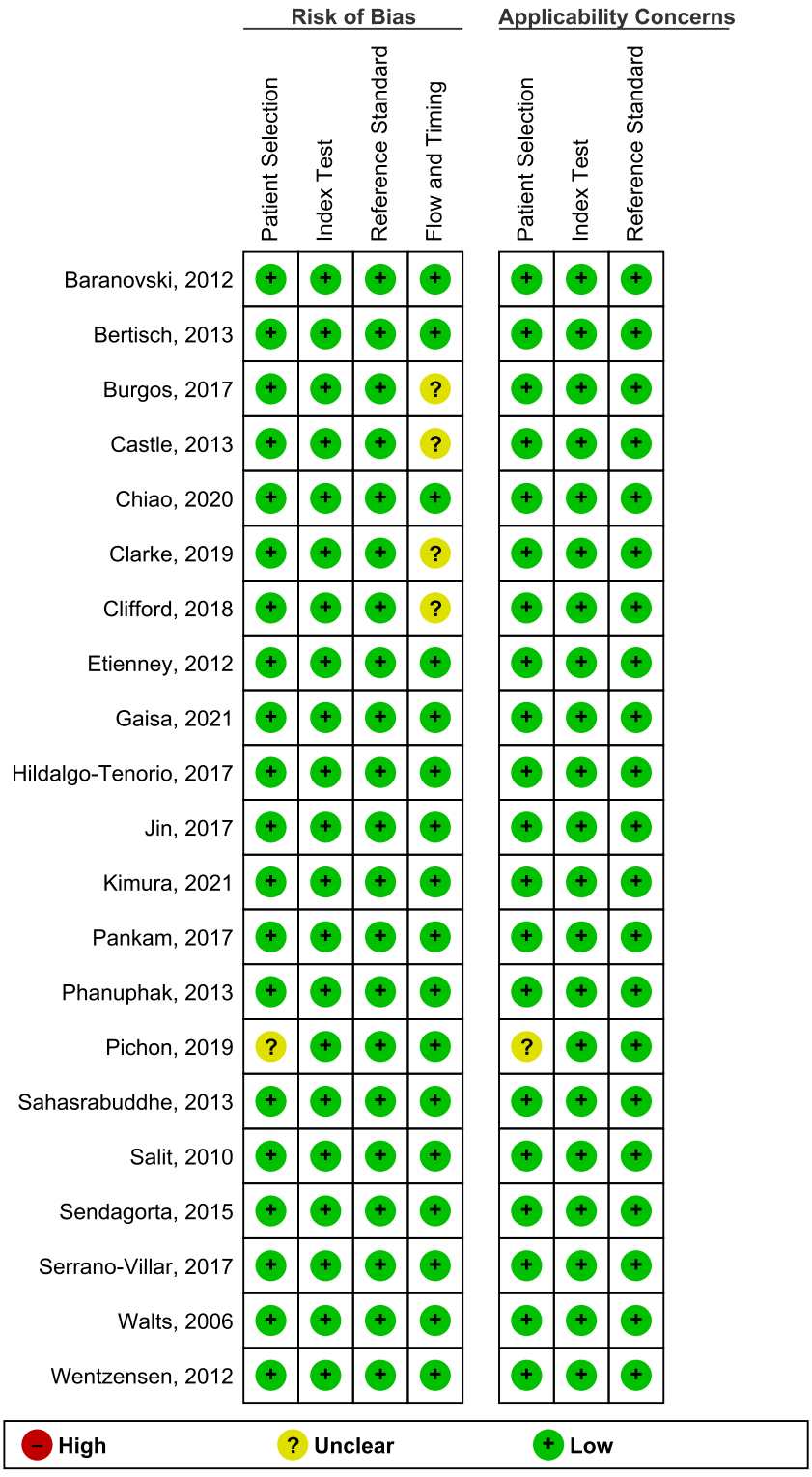

Fig. 2: **Risk of bias and applicability concerns graph.**

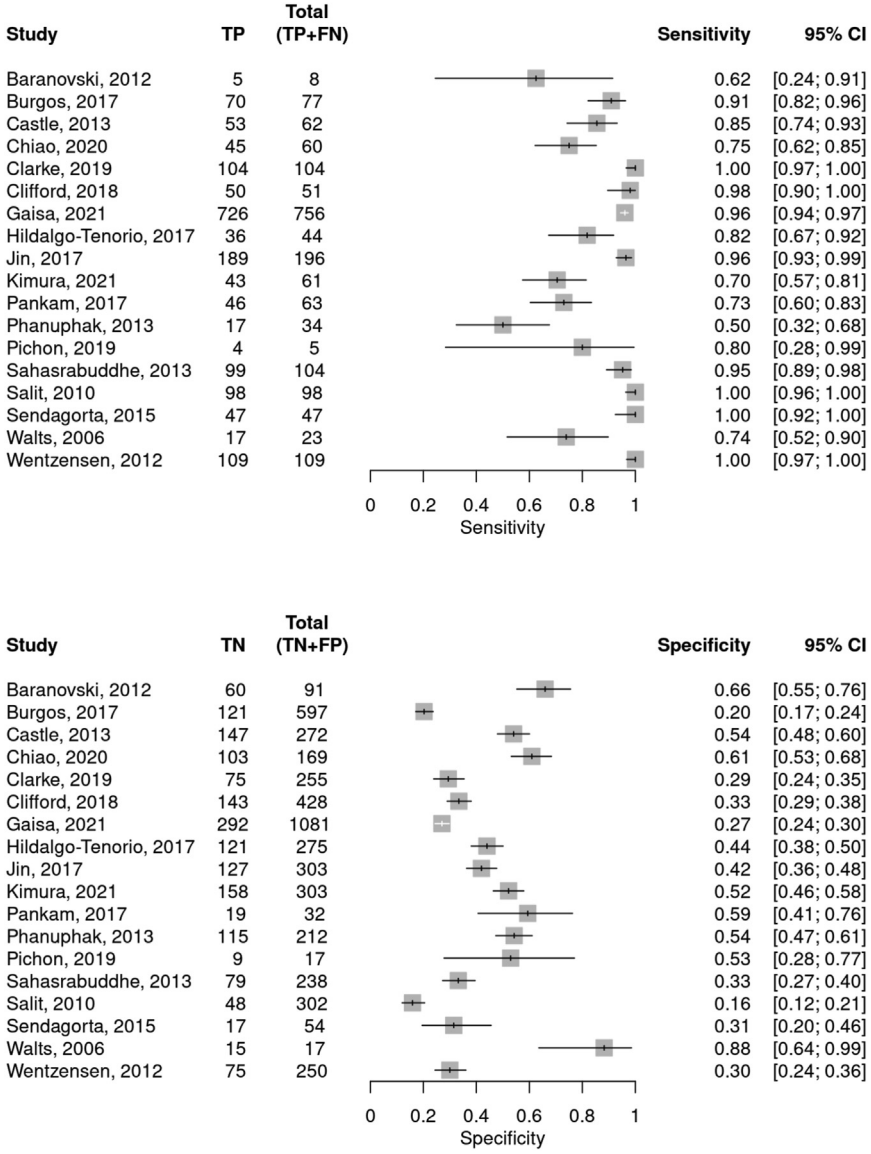

Fig. 3: **Forest plot of main analysis (test: HR DNA HPV, sample: "all together" patients, outcome: AIN1- vs. AIN2+).**

Eight studies presented HPV tests for HPV16 separately, with no majority of techniques. Three of them presented DNA HPV16 performance associated with DNA HPV18.[24,33,35]

Sensitivity analysis

We performed sensitivity analyses to validate the credibility of outcomes and investigate sources of heterogeneity. The heterogeneity of studies and its impact in the polled analysis was done by deleting individual studies and observing the impact in the results or separating in subgroups, according to.

1. Complete or partial verification.

Discerning by complete verification of the reference test or partial verification, we identified that all patients were biopsied in 12 studies, whereas in 4 studies[24,25,27,28] they were not (Table 1). In the completely biopsied sample group, the performance was similar to the "all together" results: the pooled sensitivity of DNA HR HPV was 94.6% (95% CI 94.6–94.6) and the pooled specificity was 39.7% (95% CI 39.5–39.8). The DOR was 11.537 (95% CI 11.42–11.65).

2. Different assays in the same "group tests".
Analyses were performed discriminating by assay for DNA HR HPV but no significant difference

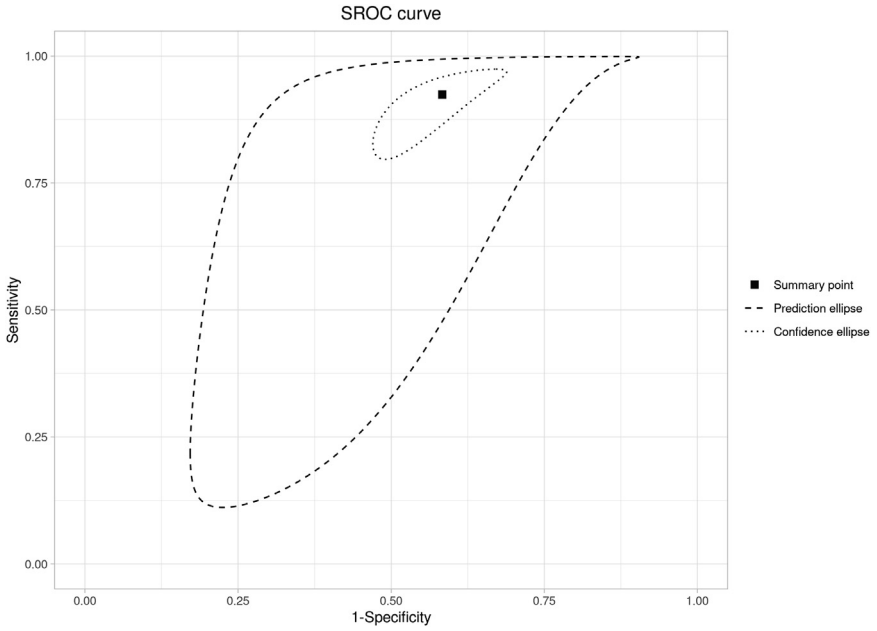

Fig. 4: **SROC Curve of main analysis (test: HR DNA HPV, sample: "all together" patients, outcome: AIN1- vs. AIN2+)**. Footnote: HSROC: hierarchical summary receiver–operator curve; AUC: area under the curve; SE (AUC): Standard Error (AUC); SE (Q*): Standard Error (Q*).

was identified (data not shown, available with authors). For mRNA HPV, comparing with and without Chiao, 2020, that used Aptima®, sensitivity was from 77.3% (95% CI 73.2–80.9) to 77.5% (95% CI 73.0–81.4) and specificity from 61.9% (95% CI 56.6–66.9) to 61.1% 61.1% (95% CI 55.1–66.6), respectively. As previously cited, this brand uses a different technique.For p16, a subanalysis of only studies that used the *CINtec PLUS* assay® was performed and showed discreetly higher sensitivity of 76.5 (95% CI 51.9%–90.8%). and lower specificity 58.4% (95% CI 47.7%–68.4%), DOR 4.57 (95% CI 1.28–7.86).[27,28,32,36,40,42]

3. Moderate risk of bias.
   Subanalysis excluding studies assessed to have moderate risk of bias did not showed difference (data not shown, available with authors).

4. Studies estimated as outliers based on the forest plot depicted in Fig. 3, selected by inspection.

We performed sensitivity analyses by removing studies estimated as outliers based on the forest plot depicted in Fig. 3. Outliers were selected by inspection of forest plots. Considering the "altogether" population, after each outlier study was excluded, the sensitivity and specificity were, respectively:

- For DNA HR HPV tests: before exclusions, 92.4% (95% CI 84.2–96.5) and 41.7% (95% CI 33.9–44.9); excluded Phanuphak 2013, 93.3% (95% CI 86.2–96.8) and 41.1% (95% CI 32.9–49.8); excluded Salit, 2010, 90.0% (95% CI 82.4–95.5) and 43.6% (95% CI 36.1–51.4); excluded Baranovski, 2012, 93.3% (95% CI 85.5–97.0) and 40.2% (95% CI 32.6–48.3).
- For mRNA HPV tests: before exclusions, 77.3% (95% CI 73.2–80.9) and 61.9% (95% CI 56.6–66.9); excluded Phanuphak 2013, 78.3% (95% CI 73.7–82.3) and 62.7% (95% CI 56.8–68.3); excluded Chiao, 2020, 77.5% (95% CI 73.0–81.4) and 61.1% (95% CI 55.1–66.6).
- For p16 tests: before exclusions, 49.7% (95% CI 31.3–68.1) and 74.3% (95% CI 58.6–85.5); excluded Phanuphak 2013, 54.3% (95% CI 34.9–72.4) and 73.2% (95% CI 54.4–86.2); excluded Kimura, 2021, 58.0% (95% CI 45.3–70.1) and 68.1% (95% CI 55.8–78.3).

The estimates of diagnostic test accuracy were altered meaningly only in the Kimura, 2021 study, for p16 tests, increasing sensitivity, probably because they have used in method random biopsy when there was no lesion in HRA. For DNA HR HPV there was no difference meaningly (sensitivity 93.2% and specificity 41.2%) and mRNA has not applied in this study.

Quantitative heterogeneity analyses

The different heterogeneity measures showed high variability. Variances of logit sensitivity and specificity ranged from 0.011 (mRNA HPV tests) to 2.708 (DNA HR HPV tests) and from 0.473 (DNA HR HPV tests) to 0.901 (DNA HPV16 tests), respectively. The I^2 bivariate

ranged from 0.700 to 0.920, and the area of the 95% prediction ellipse ranged from 6.0% (mRNA HPV tests) to 62.9% (DNA HR HPV tests). Data available in Supplement 4 table.

Investigation of publication bias

Deeks' test for funnel plot asymmetry was evaluated and showed that there were not publication bias, p value ranged from 0.1501 (mRNA HPV tests) to 0.7755 (DNA HR HPV tests). Data available in Supplement 5 table.

Discussion

The major aim of cancer screening is to reduce cancer-specific mortality. To achieve this, the incidence of cancer in the screened population must be sufficiently high, a screening test needs to be sufficiently accurate and acceptable to patients and there must be an effective intervention that is well tolerated.[46]

Anal HSIL progression varies substantially according to host factors, most notably immune status. A previous meta-analysis estimated a progression rate for anal HSIL nearly 10-fold lower in MSM without HIV infection than in MSM living with HIV.[5]

The present systematic review substantiates biomarker accuracies for anal HSIL (AIN2+) and cancer screening and confirmed that anal lesion screening follows the same trends as cervical lesion screening. As in cervical samples in previous studies, DNA HR HPV tests presented a high sensitivity of 92.4% (95% CI 84.2%–96.5%) but a very low specificity, of 41.7% (95% CI 33.9%–44.9%), suggesting that they could be a good option for screening if followed by a higher specificity test. When considering only MSM living with HIV, this disparity is quite larger: 96.8% (95% CI 89.2%–99.1%) and 32.1% (95% CI 26.3%–38.6%), respectively. These results resemble a recent review by Clarke and colleagues.[47] Only looking for understand why DNA HR HPV test has a low specificity, is necessary to take note of its feature: the test searches for virus presence and not HPV lesion signal. Because most AIN1 lesions are self-limited and evolve to cure, they are pooled as "benign". In this case, the test is properly indicated as positive (virus presence) but it is considered false positive, since AIN1 is not considered a true cancer precursor. Therefore, in the present study, as an additional analysis, when excluding AIN1 from the analysis (normal vs. AIN2+), DNA HR HPV sensitivity was 88.0% (95% CI 87.9%–88.1%) and the specificity was 61.8% (95% CI 61.6%–62.0%). In this hypothetical scenario, specificity would be much higher.

For the main analysis, mRNA HPV tests presented better performance, AUC 0.78, sensitivity 77.3% (95% CI 73.2%–80.9%) and specificity 61.9% (95% CI 56.6%–66.9%). When considering only MSM HIV+, performance slightly improved (Table 2). Good performance and better specificity of mRNA HPV tests make them a promising option. A systematic review that analysed mRNA HPV test accuracy for cervical cancer discerning by technique, identified that Aptima® (Hologic Gen-Probe, San Diego, CA, USA) exhibited superior performance than others showing, for the outcome CIN2+, a sensitivity of 92.8% (95% CI 91.9%–93.7%), near to DNA HR HPV tests, and a specificity of 60.5% (95% CI 59.8%–61.3%).[15] Unfortunately, only one study used it in present study.

Completing the outlook of the present study, p16 immunoassaying presented a good performance, with a main analysis AUC of 0.74, with near sensitivity and specificity, of 68.8% (95% CI 47.9%–84.1%), and 64.1% (95% CI 51.0%–75.4%), respectively. The DNA HPV16 test presented higher specificity for the main analysis with lower sensitivity, 71.7% (95% CI 55.3%–83.8%) and 53.3% (95% CI 35.4%–70.3%) respectively, which is understandable considering that this is the most prevalent genotype in the prevaccine era, approximately 80.7% for anal cancer, and a high oncogenic potential.[8]

One of the most promising algorithms is in effect primary screening with the DNA HR HPV test, which has superior sensitivity, and use of the mRNA HPV test, due to its high specificity and the possibility to perform the test with the same sample without the need for patient return (sample can be collected in liquid-based cytology device).[15]

Despite HPV infection, HIV infection and progression to cancer are influenced by sexual behavior, and no information about gender was described. Although it would be desirable for a qualitative analysis, we believe that it would not bias quantitative data, since all the different genders would be pooled in two groups to allow for the quantitative analysis. Unfortunately, the lack of information about screening in women could not be solved because of the shortage of studies with this sex.

Substantial heterogeneity in sensitivity and specificity was found among studies, as expected for Diagnostic Systematic Reviews (DTA handbook).[48] This could be explained by different samples and different frequencies of AIN in each population. Considering the measure of the total between-study variability, mRNA HPV tests showed the smallest area of the 95% prediction ellipse, 6.0%, influenced by the low logit sensitivity, 0.011. All other groups of tests exceed 50% prediction ellipse area, which represent a high heterogeneity.[49] We performed sensitivity analysis using different screening criteria and studies with partial or complete verification of the reference test, to try and detect confounding factors, but the results retained high heterogeneity (data not shown). Another limitation of this systematic review is the lack of primary studies with other high-risk groups, such as people living with HIV, men sex men regardless of HIV status and women with HPV. Answers for the first group are expected with divulgation of ahead ANCHOR study findings.[7]

DNA HR HPV can be a useful tool for screening for aHSIL and anal cancer if followed by a biomarker with a

higher specificity. As an isolated test, mRNA HPV had better performance, with an AUC of 0.78 for the whole group and 0.80 for MSM living with HIV.

This systematic review has shown the accuracy of DNA HR HPV, mRNA HPV, DNA HPV16 isolated and p16 staining biomarkers for anal cancer screening and allows its implementation on a larger scale. There is a strong recommendation of using DNA HR HPV for anal cancer screening if followed by a biomarker with a higher specificity for MSM living with HIV and other HIV people living with HIV. At this point of knowledge, there is a weak, or conditional, recommendation for use in other high-risk groups. Thus, additional prospective studies are necessary to establish the efficacy of cancer prevention and cost-effectiveness, leading to the definition of screening guidelines.

Contributors

Ana Cristina Macedo (ACM) was responsible for literature search, figures, study design, data collection, qualitative and quantitative data analysis, data interpretation, writing, publishing.

Antônio José Grande (AJG) was responsible for Softwares data analysis, writing.

Tatiana Figueiredo (TF) was responsible for literature search, data collection, qualitative data analysis.

Tamy Colonetti (TC) was responsible for literature search, figures, data collection.

João Carlos Gonçalves (JCG) was responsible for study design, data collection, data analysis.

Eduardo Testoni (ET) was responsible for literature search, figures, publishing.

Maria Inês da Rosa (MIR) was responsible for writing and supervision.

Therefore, ACM, AJG and JCG have verified the underlying data.

All authors had full access to all the data in the study and accept responsibility for the decision to submit for publication.

Data sharing statement

Data extract for 2 × 2 tables are discriminated by study in supplementary material for most analyses. Any other data will be available beginning immediately and ending 5 years following article publication by contacting the author by email: analacerda1979@yahoo.com.br.

Declaration of interests

The present study and authors have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102128.

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